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Oxidative stress, osmotic stress and apoptosis: Impacts on sperm function and preservation in the horse☆

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Abstract

Oxidative stress is an important component of the cytopathology of equine spermatozoa undergoing storage as liquid or frozen semen. Damage to chromatin, membranes and proteins of sperm are important components of oxidative damage to sperm. Similarly, sperm are exposed to a variety of osmotic stresses during storage that result from exposure to hypertonic media or result as a consequence of osmotic changes induced during freezing. A number of changes induced during processing and storage of equine sperm also appear to induce apoptotic-like changes which may adversely affect sperm survival and function. These processes appear in many cases to be interrelated, and this review will examine current understanding of these processes on the equine sperm function.

Keywords: Equine; Sperm; Oxidative stress; Osmotic stress; Apoptosis

1. Introduction

Although considerable progress has been made over the past few years in liquid or frozen storage of equine sperm, there remains a large inter-individual difference in the success of semen preservation for the stallion. During low temperature storage equine sperm are subjected to oxidative damage to membrane phospholipids, proteins and chromatin. Osmotic stress leads to damage

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to the plasma membrane and alteration in sperm metabolism. Furthermore, evidence from a number of species also suggests that ejaculated sperm undergo apoptotic-like changes as a consequence of cryopreservation. It appears likely that these three processes are interlinked and may impact various compartments in the sperm cell via similar pathways. Therefore, an understanding of these processes and their common metabolic pathways may be important in attempts to obviate adverse affects on equine sperm during storage.

2. Oxidative stress

2.1. General impacts

Oxidative stress is a well-defined component of many disease processes. The generation of reactive oxygen species (ROS) may occur as a normal consequence of oxidative metabolism or may result from specific mechanisms within particular cell types, such as the oxidative burst of leukocytes. Oxidative stress was suggested as an important factor in disruption of sperm function over 60 years ago (MacLeod, 1943); however, it is only recently that the importance of oxidative stress in normal and abnormal sperm function has become more apparent. It is now clear that ROS have an important role in normal sperm function and that an imbalance in either the production or degradation of ROS may have serious adverse effects on sperm. The effects of oxidative stress are particularly important during sperm storage by either cooling or cryopreservation, and this damage is further increased in situations where much of seminal plasma is removed from a semen sample because much of the antioxidant capacity in semen resides with seminal plasma.

2.2. ROS scavengers in equine semen

The primary ROS scavengers described in semen are catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx). There appears to be a wide species variation in the relative abundance and importance of these scavengers in seminal plasma, and we determined the relative activities of these enzyme systems for equine semen. The activities of catalase (98.7 ± 29.2 U/mg protein), SOD (29.15 ± 6.64 U/mg protein) and GPx (0.87 ± 0.06 μM NADPH oxidized/min/mg protein) were determined in equine seminal plasma (Ball et al., 2000; Baumber and Ball, 2005). Specific activity of catalase in tissue homogenates was significantly greater in the prostate gland than in the ampulla, bulbourethral gland, vesicular gland, cauda epididymal fluid, or testis. These data indicate that equine seminal plasma has a relatively greater activity of both catalase and superoxide dismutase with a significant variation between stallions in the activities of these scavengers. Sperm appear to have very limited amounts of ROS scavengers, and seminal plasma is a potent source of ROS scavengers which functions to protect ejaculated equine sperm from the adverse effects of ROS. The removal of seminal plasma during semen processing may increase the susceptibility of sperm to oxidative stress because of the removal of these enzyme scavengers.

In addition to the enzyme scavengers discussed above, a number of other components of seminal plasma likely counteract oxidative stress in neat semen. In particular, factors such as albumin, urate, taurine, hypotaurine, pyruvate, lactate, ascorbic acid, tocopherol and ergothioniene are present in seminal plasma and may act as antioxidants (Mann et al., 1963; Alvarez and Storey, 1983; De Lamirande and Gagnon, 1992; Halliwell and Gutteridge, 1999). Relatively less research has been conducted on the composition of equine seminal plasma and the role of low molecular weight antioxidants in equine semen; although assessment of the total antioxidant capacity of